Computational Prediction of Bacterial Behavior

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Can we use a computational model to predict the response of a microbe to new environmental signals? The answer to this question is important—in part because the ability to make accurate predictions would vastly reduce the experimental effort required to harness microbes for biofuels and carbon management. To better understand the technical challenges involved, we investigated this question for a well-characterized system: induction of lactose utilization genes from the *lac* operon in Escherichia coli [1].

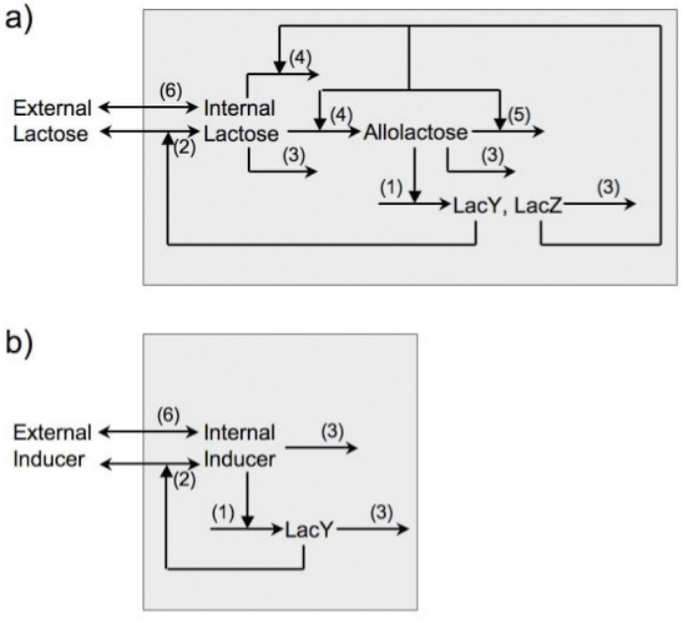

Fig. 1. Models of lac induction. (a) Model for lac induction by lactose, including the following processes: (1) proportional production of permease (LacY) and β-gal (LacZ), (2) permease-mediated transport of lactose, (3) dilution of intracellular species by cell growth, (4) β-gal catalyzed degradation of lactose, producing both the metabolic intermediate allolactose, and the ultimate products of degradation, glucose and galactose, (5) β-gal catalyzed degradation of allolactose, producing glucose and galactose, and (6) passive transport of inducer. (b) Induction by artificial inducers, including: (1) proportional production of permease (LacY) and β-gal (LacZ), (2) permease-mediated transport of inducer, (3) dilution of intracellular species by cell growth, and (6) passive transport of inducer.

In 1957, Novick and Weiner [2] studied expression of the *lac* operon in response to an artificial inducer, thio-methylgalactoside (TMG), which is not degraded by the induced enzyme β-galactosidase (β-gal). They discovered that this system can exhibit discontinuous switching, with some cells expressing a large amount of enzyme, other cells expressing a small amount, and an insignificant number of cells expressing an intermediate amount. Recently, this effect was further characterized using single-cell assays of fluorescence levels in a population of *E. coli* cells carrying a *lac::gfp* reporter [3]. Cells were grown overnight on sucrose in either an induced (1 mM TMG) or uninduced (no TMG) state. They were then diluted into media with defined levels of TMG and glucose. After 20 hours of growth, the cells were examined under a microscope. Under many conditions, cell populations exhibited a bimodal distribution, with induced cells having over 100 times the fluorescence level of uninduced cells. The distribution was also history-dependent—at the same final level of TMG and glucose, cells with an induced history were predominantly induced, while cells with an uninduced history were predominantly uninduced. These observations have been attributed to the existence of two steady states, i.e., bistability, in the induction of *lac* in *E. coli.*

The aim of our study was to predict whether *lac* induction would also exhibit bistability in response to a natural inducer (lactose) that is degraded by enzyme. To address this question, we first developed a model of *lac* induction in response to TMG (Fig. 1b). The model is applicable in the absence of external glucose, which is a physiologically relevant condition for lactose utilization by *E. coli*. The model was constrained using a large body of biophysical data from the literature, and was validated using the published data of Ozbudak et al. [3]—it exhibited bistability for a range of TMG levels that is consistent with the data (Fig. 2).

We then modified the model to consider induction by lactose (Fig. 1a). The model exhibited no bistability within the constraints from available biophysical data (Fig. 3). Further analysis of the model yielded predictions of specific factors that can promote bistability, such as hindering enzyme

kinetics, suggesting potential targets for engineering mutants that are bistable in response to lactose.

Our results are consistent with preliminary experimental studies of lactose induction of the *lac* operon [3], which showed no bistability. They emphasize the importance of metabolic fluxes in determining microbial responses, and suggest that it might be possible to predict microbial behavior in response to new environmental signals. Using our methods, we were able to predict genetic targets for engineering mutants that are bistable in response to lactose. Similar methods might be useful for optimizing microbial behavior to produce biofuels and manage carbon flow.

For further information contact Michael E. Wall at mewall@lanl.gov.

[1] D.W. Dreisigmeyer et al., *IET Syst. Biol.* **2,** 293 (2008).
[2] A. Novick and M. Weiner, *Proc Natl Acad Sci USA* **43,** 553 (1957).
[3] E.M. Ozbudak et al., *Nature* **427,** 737 (2004).

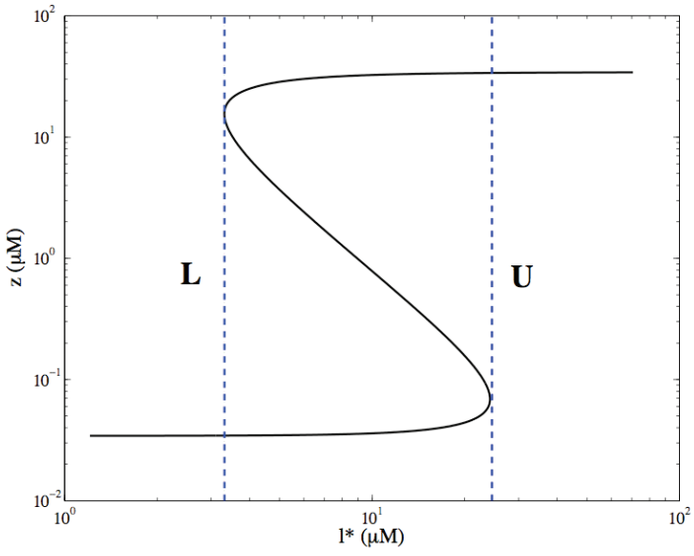

Figure 2. Simulated induction of lac by TMG. At external TMG levels (x axis) between the upper (U) and lower (L) turning points, there are three steady-state expression levels (y axis): a high level, which is stable, an intermediate level, which is unstable, and a low level, which is stable. The system is therefore predicted to be bistable for TMG levels between U and L—the range shown here is consistent with that in [3].

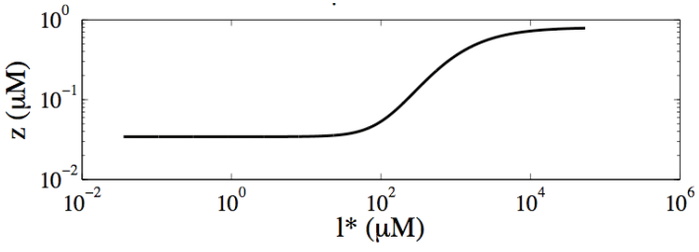

Figure 3. Simulated induction of lac by lactose. At all external TMG levels (x axis), there is a single steady-state expression level (y axis). The system is therefore not bistable.

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